# EXTRACTION OF ANTIOXIDANT, PHENOLIC CONTENT AND MINERALS OF COLEUS *BLUMEI* LEAVES BY BOILING

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I declare that this thesis entitled "*Extraction of antioxidant, phenolic content and minerals of Coleus blumei leaves by boiling*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : .....
Name of Candidate: NORHIDAYAH BINTI HAJI SAAT
Date : 20 APRIL 2009

Special Dedication of This Grateful Feeling to My…

***Beloved father and mother;***
***Mr. Haji Saat bin Haji Ahmad and Mrs. Maznah bt Jais***

***Loving brothers and sisters;***
***Mohd Nizam, Norzira, Nora'ain and Mohd Najib***

***Supportive families;***
***Uncles and Aunties***

For Their Love, Support and Best Wishes.

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## ABSTRACT

Coleus leaves are commonly known as ati-ati leaves in Malaysia. Previous study has shown that the Coleus leaves have high antioxidant activity and nutritional value. The present work is to investigate whether antioxidant, minerals and phenolic content can be extracted by boiling the leaves in water. The antioxidant was determined by mixing the extract solution with DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) solution using different ratios. Acid ascorbic acid was used as standard in measurement by Uv-Vis Spectrophotometer. Phenolic content was measured by Uv-Vis Spectrophotometer using Gallic acid as standard. There is about 40.77 wt % of antioxidant activity, 6.256998 wt% of total phenolic content, and some minerals (magnesium, calcium, iron and zinc) existing in solution after the Coleus *blumei* leaves were removed. The wt% of the phenolic content is directly proportional to the wt% of antioxidant activity. The mineral concentration, antioxidant activity and phenolic content seemed to be highly correlated. As a conclusion, it is proven that the Coleus *blumei* leaves have high potential value for the nutritional purpose.

# ABSTRAK

Daun *Coleus* dikenali sebagai daun ati-ati di Malaysia. Kajian sebelum ini menunjukkan bahawa daun Coleus menpunyai nilai antioksida dan nilai nutrisi yang tinggi. Kajian terkini ialah untuk mengkaji sama ada antioksida, kandungan fenol dan mineral boleh diekstrak dengan merebus daun ke dalam air. Antioksida dicari dengan mencampurkan larutan ekstrak dengan larutan *DPPH (2,2-Diphenyl-1-Picrylhydrazyl)* menggunakan nisbah yang berbeza. Asid askorbik digunakan dalam pengukuran menggunakan *Uv-Vis spectrophotometer*. Kandungan penol diukur menggunakan *Uv-Vis spectrophotometer* di mana acid galik digunakan sebagai larutan pengukur. Terdapat lebih kurang 40.77 wt% (peratus berat) bagi antioksida, 6.256998 wt% bagi kandungan fenol , dan beberapa mineral (magnesium, kalsium, zat besi , dan zink ) wujud dalam larutan setelah daun *Coleus blumei* dibuang. Peratusan berat kandungan fenol berkadar langsung dengan kandungan antioksida. Kepekatan mineral, antioksida dan kandungan fenol sangat berkait rapat antara satu sama lain. Sebagai kesimpulan, daun *Coleus blumei* terbukti mempunyai potensi dan nilai nutrisi yang tinggi.

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# CHAPTER 1

# INTRODUCTION

## Introduction

With more health conscious society nowadays; the demand for healthier food product has been increasing. Fruits and vegetable provide important health benefits in our daily life. Nowadays, herbs are widely used to provide important health benefits in human diet. Herbs also believed have protective effects on high level exposure of free radicals that can cause the damage of cellular. Free radical can cause many diseases and can contribute to the aging process (Ames et. Al, 1993).The harmful action of the free radicals however can be blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism. Current research has confirmed that food rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancer (Gerber et al., 2002; Kris-Etherton et al., 2002; Serafini, Bellocco, Wolk, & Ekstrom, 2002) as well as inflammation and problems caused by cell and coetaneous aging (Ames, Shigrenaga, & Hagen,1993). Antioxidants also can control the degenerative diseases where the oxidative damage has been implicated. Several plant extracts and different classes of phytochemicals have been shown to have antioxidant activity (Al Saikhan, Howard, & Miller, 1995; Bergman, Varshavsky, Gottlieb, & Grossman, 2001; Cao, Sofic, & Prior, 1996; Oomah & Mazza, 1994; Wang, Cao, & Prior, 1996; Yen & Duh, 1995). The search for newer natural antioxidants, especially of plant origin, has ever since increased. There are three important aspects that we focus

on this research which are determination of minerals, anti antioxidant activity and phenolic content.

## 1.1 Research Background / Problem Statement

In medical production, many researchers do a lot of efforts in order to improve the quality of supplementary food. Nowadays, mostly supplementary food is made by using chemical. This also brings out some effects for our health and bodies. So they try to find any other methods to produce better product like using the herbal and traditional methods. In fact of producing the traditional product gives a lot of advantages for our health using the traditional methods, there also have some weaknesses. The liquid solution form usually cannot be kept for a long time period. Using the preservative can damage the pureness of the herbs and lower the effectiveness and its quality. Thus, production of herbal supplementary food into solution form is not really suitable for commercial.

## 1.2 Objectives

The proposed research is aimed at determining the mineral profile, phenol content, and antioxidant activity in water that has been used to boil Coleus leaves.

## 1.3 Scopes of Study

To achieve the objectives, there are some scopes have been identified in this research:

i. Study on how to measure antioxidant and phenolic content in the solution of boiled coleus using UV/visible spectrophotometer
ii. Study on how to determine mineral profiles in the solution of boiled coleus using AAS

## 1.4 Rationale and Significance

Since the discovery of using Coleus in food supplement is not really well known yet, therefore it is expected that the information and knowledge gained from this research studies will increase the awareness of using this traditional plant hence provide much optional treatments to cure any diseases related.

# CHAPTER II

# LITERATURE REVIEW

## 2.1 Definition of Coleus

Coleus is a name which derives from an earlier classification under the genus name *Coleus*, species of which are currently included in either *Solenostemon* or another genus, *Plectranthus*. The word Coleus come from the Greek “koleus’, meaning sheath. It is believed that there are 150 species of Coleus .It is a genus of perennial plants, native to tropical Africa, Asia, Australia, the East Indies, the Malay Archipelago, and the Philippines. Many cultivars of the Southeast Asian species *Coleus* have been selected for their colorful variegated leaves, usually with sharp contrast between the colors where the leaves are green, pink, yellow, maroon, and red. Typically, in Malaysia this plant known as ati-ati. The plants need a well condition of in moist-drained soil to grow, and typically grow 0.5-1 m tall, though some may grow as tall as 2 meters. They are heat-tolerant, though they do less well in full sun in subtropical areas than in the shade. The leaves of the green type are often eaten raw with bread and butter. The chopped leaves are also used as a substitute for sage (Salvia officinalis Linn.) in stuffing. C. aromaticus is used for seasoning meat dishes and in food products (Uphof, 1959) while a decoction of its leaves is administered in cases of chronic cough and asthma (CSIR, 1992). It is considered to be an antispasmodic, stimulant and stomachic and is used for the treatment of headache, fever, epilepsy and dyspepsia (Khory &Katrak, 1999; Morton, 1992).

## 2.2 Antioxidant

An antioxidant in food is really important as it can protect human body from free radicals activity. It is also has capable of slowing or preventing the oxidation of other molecules. When electrons are transferred form a substance to an oxidizing agent, it called as oxidation reaction. Free radicals can be produced during the Oxidation reactions, where the start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols.

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells.

As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease.

Although some studies have suggested antioxidant supplements have health benefits, other large clinical trials did not detect any benefit for the formulations tested, and excess supplementation may be harmful In addition to these uses in medicine, antioxidants have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.

Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers. As far as our literature survey could ascertain, antioxidant activities of this plant have not previously been published.

Hence, the previous work investigated the possible antioxidative effects of freeze-dried powder obtained from aqueous extract of fresh leaves of *C. aromaticus*. In this study, they had examined the antioxidant activity of CAE (*C.aromaticus* hydroalcoholic extract) employing various in vitro assay systems, such as the β-carotene-linoleate model system, DPPH (2,2-Diphenyl-1-Picrylhydrazyl)/superoxide/ nitric oxide radical scavenging, reducing power and iron ion chelation, in order to understand the usefulness of this plant as a foodstuff as well as in medicine.

### 2.2.1 Antioxidant Assay using a *β*-carotene-linoleate Model System

On the previous experiment, the antioxidant activity of the extract was measured by the bleaching of β-carotene. By adding CAE and BHT (Butylated Hydroxytoluene) at various concentrations, it can prevent the bleaching of β-carotene to different degrees. β-Carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. The linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, which attacks the highly unsaturated β-carotene molecules. As a result, β-carotene will be oxidized and broken down in part; subsequently, the system looses its chromophore and characteristic orange colour, which can be monitored spectrophotometrically. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system (Jayaprakasha, Singh, & Sakariah, 2001).

It also showed that the CAE was found to hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system. In comparison, the CAE showed an appreciable antioxidant activity of 83.0% at 250 µg/ml, while BHT, a synthetic antioxidant had 89.6% antioxidant activity at 100 µg/ml.

**Table 2.1**: Antioxidant activity of aqueous extract of C. aromaticus in β-carotene-linoleate system

| Sample | Concentration (µg/ml) | Antioxidant activity (%) |
|---|---|---|
| Aqueous extract | 125 | 53.2 ± 1.04 |
| | 250 | 83.0 ± 1.33 |
| | 500 | 91.3 ± 1.41 |
| BHT | 50 | 64.2 ± 1.81 |
| | 100 | 89.6 ± 1.52 |
| | 200 | 95.3 ± 1.33 |

### 2.2.2 DPPH Radical-scavenging Activity

The CAE showed a concentration-dependent antiradical activity by inhibiting DPPH radical with an $EC_{50}$ value of 210 µg/ml (Table 2). DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants (Oyaizu, 1986). The method is based on the reduction of methanolic DPPH solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The extract was able to reduce the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (e.g., hydroquinone, pyrogallol, gallic acid), and aromatic amines (e.g., *p*-phenylene diamine, *p*-aminophenol), reduce and decolorise 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability (Blois,

1958). It appears that the CAE possesses hydrogen donating capabilities and acts as an antioxidant. The scavenging effect increased with increasing concentration of the extract. However, scavenging activity of Gallic acid, a known antioxidant, used as positive control, was relatively more pronounced than that of CAE.

**Table 2.2**: Antiradical activity of aqueous extract of *C. aromaticus* observed with DPPH

| Sample | Concentration (µg/ml) | % Inhibition | EC50 (µg/ml) |
|---|---|---|---|
| Aqueous extract | 60 | 11.3 ± 0.22 | 210 |
| | 120 | 27.0 ± 0.41 | |
| | 180 | 42.0 ± 1.79 | |
| | 240 | 58.4 ± .050 | |
| | 300 | 72.7 ± 0.33 | |
| Gallic acid | | | 1.38 |

### 2.2.3 Assay of Superoxide Radical ($O_2^-$)-Scavenging Activity

The superoxide radical ($O_2^-$)-scavenging activity of the extract, were previously measured by the riboflavin-NBT-light system in vitro. Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species (Halliwell & Gutteridge, 1985). Photochemical reduction of flavins generates $O_2^-$, which reduces NBT, resulting in the formation of blue formazan (Beauchamp & Fridovich, 1971). The extract was found to be a moderate scavenger of superoxide radical generated in riboflavin-NBT-light system in vitro. The extract inhibited the formation of the blue formazan and the % inhibition was proportional to the concentration with an EC50 value of 73.9 µg/ml. These results indicated that the tested extract had a notable

effect on scavenging of superoxide when compared with ascorbic acid, which was used as positive control.

**Table 2.3**: Superoxide anion-scavenging activity of aqueous extract of *C. aromaticus* observed with the riboflavin-light-NBT system

| **Sample** | **Concentration (µg/ml)** | **% Inhibition** | **$EC_{50}$ (µg/ml)** |
|---|---|---|---|
| Aqueous extract | 25 | 13.3 ± 1.89 | 73.9 |
| | 50 | 35.4 ± 1.14 | |
| | 75 | 52.5 ± 1.30 | |
| | 100 | 66.5 ± 1.05 | |
| Ascorbic acid | | | 17.4 |

### 2.2.4. Assay of Nitric Oxide-Scavenging Activity

The extract also showed a moderate nitric oxide-scavenging activity between 25 and 200 µg/ml in a dose-dependent manner ($EC_{50}$ = 173 µg/ml) (Table 4). In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Moncada, Palmer, & Higgs, 1991). The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health. The extract showed a moderate nitric oxide-scavenging activity. The % inhibition was increased with increasing concentration of the extract. Curcumin, a natural antioxidant was used as a positive control for comparison (Sreejayan & Rao, 1997).

**Table 2.4**: In vitro NO-scavenging activity of aqueous extract of *C. aromaticus*

| **Sample** | **Concentration (µg/ml)** | **% Inhibition** | **$EC_{50}$ (µg/ml)** |
|---|---|---|---|
| Aqueous extract | 25 | 14.4 ± 1.08 | 173 |
| | 50 | 20.2 ± 0.79 | |
| | 100 | 35.1 ± 0.77 | |
| | 200 | 55.6 ± 1.02 | |

DPPH radical-scavenging activities and amount of the isolated compounds of Coleus aromaticus are showed in Table 2.4 and 2.5. Values of tested material in Table 2.5 were determined from integration of HPLC signals and response factors calculated from standards. The results are from three separate experiments.

**Table 2.5**: Tested materials EC50 (lg/ml ± SD) Amounts (mg/ga ± SD)

Hexane extract >500 –

Ethyl acetate extracts 84.0 ± 0.35 –

Aqueous extract 348 ± 2.46 –

Chlorogenic acid 11.0 ± 0.57 1.33 ± 6.58

Rosmarinic acid 9.96 ± 0.94 44.8 ± 1.84

Caffeic acid 5.52 ± 0.35 2.42 ± 1.84

Gallic acid 1.38 ± 0.22 –

Figure 2.1 shows the extraction scheme for the isolation of antioxidant compounds from Coleus aromaticus while Figure 2.2 showed the structure of Compounds isolated from Coleus aromaticus leaves.

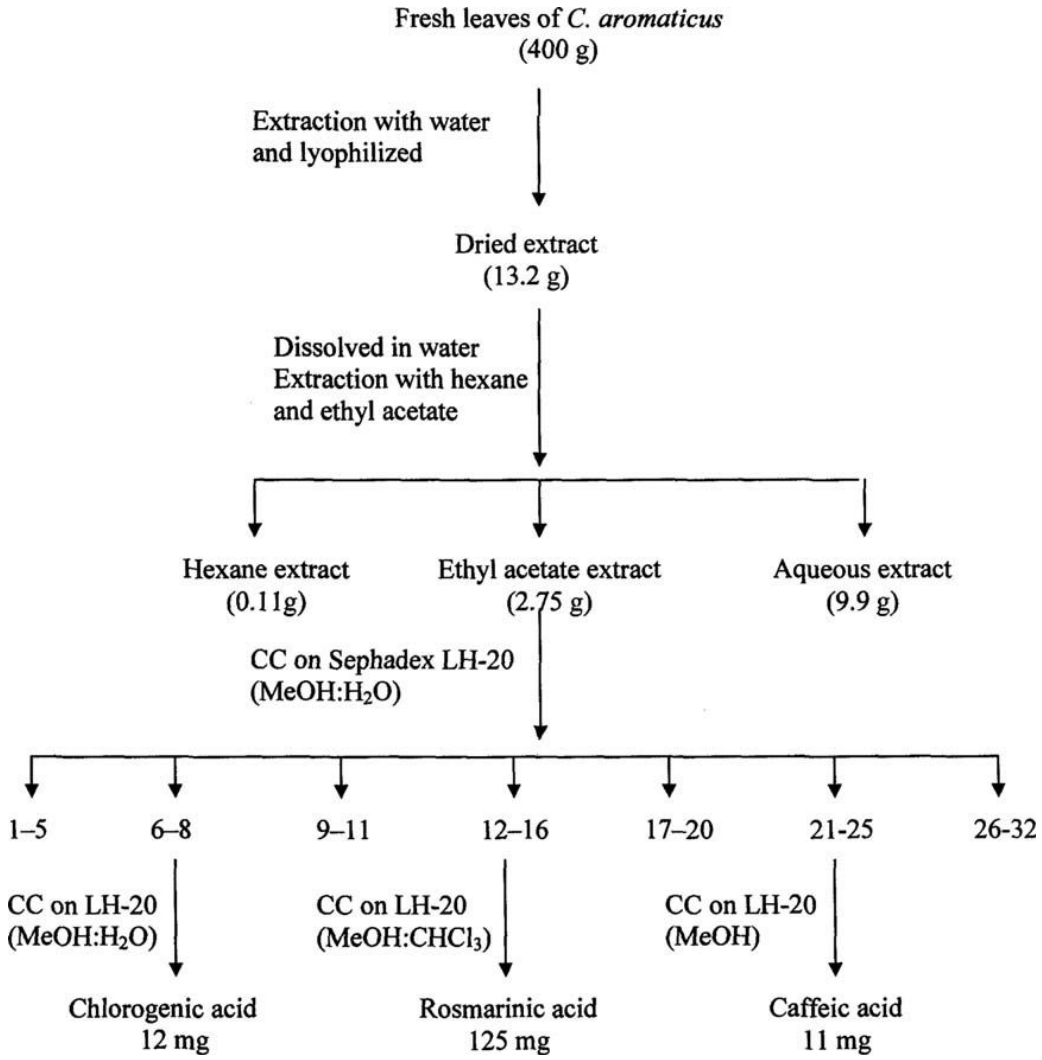


**Figure 2.1**: Extraction scheme for the isolation of antioxidant compounds from Coleus aromaticus

**Table 2.6**: Spectral data of the compounds isolated from Coleus *aromaticus*

| Name of compounds | $^1$H NMR(270 MHz) TMS as int. standard | $^{13}$C NMR(68 MHz) TMS as int. standard | FAB MS (m/z) |
|---|---|---|---|
| (1) Chlorogenic acid | 1.62–2.12 (m) 3.54–4.03 (m) 5.03–5.19 (m) 6.28 (d) 6.78 (d) 6.99 (dd) 7.06 (d) 7.57 (d) | 36.56, 37.27, 68.34, 70.65, 70.94, 73.63, 114.33,114.86, 115.75, 121.31, 125.64, 144.94, 145.57, 165.82, 175.15, | 355 [M + H]+, 377 [M + Na]+ |
| (2) Rosmarinic acid | 3.10 (2q) 5.24 (dd) 6.32 (d) 6.68 (dd) 6.77 (d) 6.87 (d) 6.88 (d) 7.05 (dd) 7.18 (d) 7.58 (d) | 37.40, 73.73 114.77,115.16, 115.95,116.33, 117.28,120.39, 121.63, 122.78 127.37, 129.09, 144.71, 145.63, 146.21, 146.64, 148.90, 166.93, 171.36 | 361 [M + H]+, 383 [M + Na]+ |
| (3) Caffeic acid | 6.77 (d) 6.96 (dd) 7.05 (d) 6.28 (d) 7.42 (d) | 114.64,115.24, 115.83,121.20, 125.80, 144.60, 145.63, 148.17, 168.00 | 181 [M + H]+, 203 [M + Na]+ |

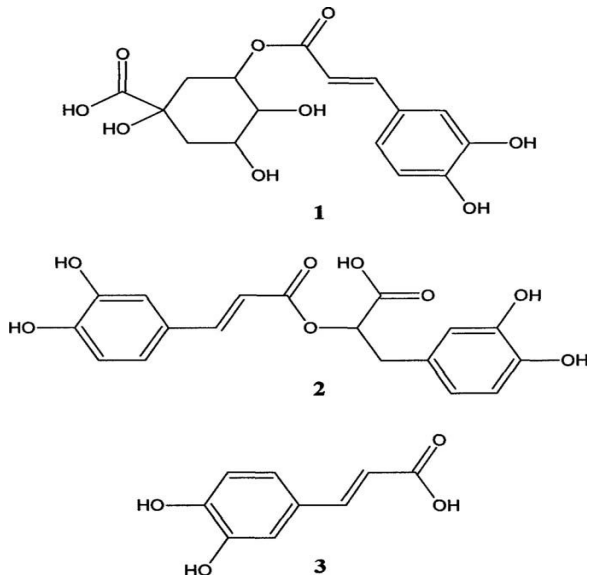


**Figure 2 2:** Compounds isolated from Coleus *aromaticus* leaves

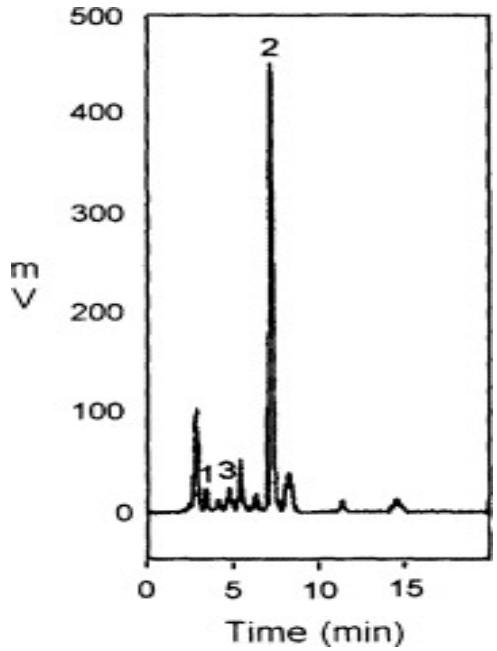


**Figure 2.3**: HPLC chromatogram of the ethyl acetate extract of *Coleus aromaticus* leaves. Chlorogenic acid (**1**), rosmarinic acid (**2**), and caffeic acid (**3**) were detected at 325 nm

The aqueous extract of *C. aromaticus* leaves exhibited different levels of antioxidant activity in all the models studied. The results from various free radical-scavenging systems revealed that the *C. aromaticus* had significant antioxidant activity and free

radical-scavenging activity which showed in Figure 2.3. The free radical-scavenging property may be one of the mechanisms by which this drug is useful as a foodstuff as well as a traditional medicine. Table 2.6 showed the spectral data of the compounds isolated from Coleus *aromaticus*. However, further investigation of individual compounds, their in vivo antioxidant activities and in different antioxidant mechanisms is warranted.

## 2.3 The UV/Vis Spectrophotometer

The UV/Vis spectrometer consists of a light source, a sample compartment, a diode-array detector, and a data acquisition computer. The sample compartment is between the light source and the detector. The spectrometer measures the amount of ultraviolet and visible light transmitted by a sample placed in the sample compartment. Typically liquid samples are used, contained in a transparent "cuvette" or "cell". A flow-through cell for the kinetics experiment is currently in the sample compartment, but another standard cuvette can easily be substituted for it. The sample compartment in our spectrometer is made for 1 cm cuvettes.

### 2.3.1 Theory of Absorption

Figure 2.4 shows the theory of adsorption of Uv/vis spectrometer while Figure 2.5 shows the Uv/vis spectrometer.When white light passes through or is reflected by a colored substance, a characteristic portion of the mixed wavelengths is absorbed. The remaining light will then assume the complementary color to the wavelength(s) absorbed. This relationship is demonstrated by the color wheel shown on the right. Here, complementary colors are diametrically opposite each other. Thus, absorption of 420-430 nm light renders a substance yellow, and absorption of 500-520 nm light makes it red. Green is unique in that it can be created by absorption close to 400 nm as well as absorption near 800 nm.